Claim 1 of the subject invention concerns a cloning method. The method involves providing a sample repertoire of nucleic acid sequences encoding immunoglobulin variable domains. The sequences are then copied and cloned using forward and back primers for expression of the repertoire of immunoglobulin variable domains. The forward primer is specific for a sequence at/adjacent the 3' end of the sense strand of the immunoglobulin variable domain nucleic acid sequences. The back primer is specific for a sequence at/adjacent the 3' end of the antisense strand of the sequences.

The claim uses the term "repertoire." Any given animal is able to make a huge number of different antibody molecules. The differences occur in the variable domains. Thus a mouse can make between 10^6 and 10^9 different antibody molecules and this range is called its antibody repertoire. However, that mouse has more types of antibodies than he has genes and there will be a smaller (but nevertheless large) repertoire at the genetic level. In particular, that mouse will have a large repertoire of primordial variable region genes (each different from the other) which may be mutated or combined with other gene segments to provide the mature antibody repertoire.

Claim 1 concerns the provision of an expression repertoire of variable domain sequences which by their very nature are inherently variable. Before the present invention, no one had

even thought of providing an expression repertoire of variable domain sequences, let alone how it might be done.

Applicants were first to (a) have the idea of providing an expression repertoire of variable domain sequences; and then first to (b) explain how to do it. The subject invention involves two levels of inventive contribution to this field.

Having first recognized the whole idea of providing an expression repertoire of variable domain sequences, the issue of how to do it was still not obvious. The inventors next contribution was one of discovering how in fact they could provide an expression repertoire of variable domain sequences. This depended upon the present invention fortuitous and surprising discovery that there is sequence conservation at the 5' end of variable domain coding sequences. This meant that the inventors could make a comparatively small general primer pool which provided a specific match for each variable domain sequence within the repertoire. The general primer pool provides a specific match for each variable domain sequence within the repertoire (i.e., not merely a different back primer specific for each member of the population). There is nothing in the prior art which establishes that the 5' end of those variable domain immunoglobulin sequences is sufficiently conserved to allow the design and use of a small general primer pool.

The skilled person would not have contemplated that the principles of PCR could be applied to a population of immunoglobulin variable domain sequences since he would know that by their very nature these sequences are variable. The skilled person would only believe that each sequence member of the population could be cloned separately using specific primers for each sequence. He would not have considered cloning a genetically diverse repertoire of immunoglobulin variable region coding sequences. Furthermore, the art does not teach that despite being variable there is sufficient sequence conservation at the 5' end for the repertoire to be cloned by use of a comparatively small general back primer pool. Applicants were the first to conclusively establish the important fact of sequence conservation at the 5' end of variable domain coding sequences.

Claims 33-63 stand rejected under 35 U.S.C. §103 as being unpatentable over Mullis et al. Reconsideration is requested.

Mullis relates to PCR for amplifying and detecting any target nucleic acid sequence in a nucleic acid or mixture of nucleic acids. There is no disclosure relating to the amplification/cloning of immunoglobulin sequences, and more importantly there is no disclosure relating to the amplification/cloning of a population of different immunoglobulin variable domain target sequences. The Examiner's

comment that novelty in the starting materials and/or in the final product does not lend patentability to art-known process of making is false where a skilled person would not believe that the process could be applied to the starting material.

Mullis makes no teaching about cloning directly for expression. Applicants in contrast are amplifying a repertoire/population of variable domain sequences with sites for direct cloning into expression vectors.

The whole thrust of teaching on PCR as exemplified by Mullis is that a tailor-made primer is necessary for each target sequence. Mullis clearly teaches at column 8, that sequencing of the target sequence must be carried out and where there is any doubt about the exact sequence, a number of slightly different primers must be made so that one of that number will be specific for the target sequences.

The PCR teachings as illustrated by Mullis lead the skilled person directly away from the present invention - amplifying/cloning a population of inherently variable sequences using a general/primer pool. The skilled person would believe that he must amplify/clone each member of the population one by one since the teachings on PCR tell him that he must use a tailor-made primer for a given member having carried out the necessary sequencing studies.

Claims 38-45 and 57-63 stand rejected under 35 U.S.C. §103 as being unpatentable Skerra et al in view of either Mullis or Herzog, and in view of Kabat et al. Reconsideration is requested.

The present invention is unobvious because the Applicants were the first to formulate the idea of providing an expression repertoire of variable domain sequences. Further their method for how this could be achieved is unobvious. As noted above, Mullis teaches away from being able to clone/express a population of sequences each different from the other using a generally back primer pool. The teaching of Herzog is no more relevant, as they used a pair of primers specific for a particular subgroup to take into account differences in HPVE7 from different virus subgroups. Thus Mullis and Herzog teach that you must have primers specific to your target sequence are necessary. Skerra simply discloses synthesizing DNA encoding both light chain and heavy chain variable domains and then expressing this DNA is *E.coli* to produce a folded Fv fragment with antigen binding capacity. Kabat lists the sequences for portions of various immunoglobulins. Kabat reference makes no suggestion that there is any degree of sequence conservation at the 5' ends of the variable domain coding sequences.

Given these teachings, how then would the skilled person clone a population of sequences each different from the other?

Mullis and Herzog would teach him that he would need primers specific for each target sequences. Based on these teachings his approach therefore would be one of isolating a first member of the population, sequencing at least part of it, designing a primer for it, carrying out PCR followed by, e.g., cloning/expression. This would be repeated for each member of the population. Kabat would only be a shortcut for the immunoglobulins listed by Kabat. The Kabat reference only provides information about amino acid and codon distribution within VH and VL domains. Kabat provides no teaching about individual nucleotides at any given position. This information would be needed in order to prepare suitable primers. Indeed the art teaches that information about individual nucleotides at a given position is a prerequisite for successful amplification by PCR. This is why all publications on this refer to sequencing of the target sequence.

There is absolutely no way a skilled person reviewing these four references would find the claims of the subject invention obvious.

Claims 34-37 and 46-56 stand rejected under 35 U.S.C. §103 as being unpatentable over Skerra in view of either Mullis or Herzog in view of Kabat et al as applied to claims 38-45 and 57-63 above, and further in view of Schoemaker et al. Reconsideration is requested.

Schoemaker disclose hetero chain antibodies consisting of heavy and light chains from different sources. Schoemaker does not supplement the deficiencies of the Skerra, Mullis, Herzog and Kabat references.

Should the Examiner maintain the above art rejections, it is requested that the Examiner indicate exactly where in these references there is disclosure or even suggestion that the 5' ends of variable domain coding sequences are sufficiently conserved to allow design and use of general back primers. Without this understanding, the skilled person would not consider cloning multiple immunoglobulin variable domain sequences as required by the method steps of the subject claims. Should the Examiner maintain his rejections, it is also requested that the Examiner indicate where in the citations there is any teaching that one can use PCR to simultaneously clone a population of variable sequences using a general primer pool.

For the foregoing reasons, Applicants submit that the claims as set forth herein are in clear condition for allowance and therefore a Notice of Allowance is earnestly solicited.

Respectfully submitted,

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